Publication I.

EFFICACY OF DIFFERENT UV EMITTING LIGHT SOURCES IN THE INDUCTION OF T CELL APOPTOSIS

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Abbreviations: broad-band ultraviolet B, BB-UVB; MED, minimal erythema dose; NB-UVB, narrow-band UVB; XeCl, xenon chloride; MECLR, mixed epidermal cell lymphocyte reaction; MLR, mixed lymphocyte reaction; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; mAb, monoclonal antibody; AD50, the energy density necessary to induce apoptosis in 50% of the T cells;

ABSTRACT

Ultraviolet B (UVB) radiation is a modality widely used for the treatment of different skin diseases. One of the major mechanisms of UVB immunosuppression in this treatment modality is thought to be an apoptosis-inducing effect on T cells infiltrating the skin. We examined the T cell apoptosis-induction capacities of 4 different UV light sources, with and without UV filters. The xenon chloride (XeCl) laser proved to be the strongest apoptosis-inducer. The use of a phtalic acid filter eliminated UV radiation almost completely below 300 nm, which resulted in a severe decrease in the apoptosis-inducing capacity of different UVB sources. Using the results of the measurements with polychromatic UV light sources, the wavelength dependence of UVB light for the induction of T cell apoptosis was also determined. The regression line of the action spectrum demonstrated a continuous decrease from 290 nm to 311 nm. The apoptosis-inducing capacity of the XeCl laser was almost 4 times higher than the calculated value according to the action spectrum, which might be due to the high irradiance of the laser as compared with non-laser light sources.

INTRODUCTION

Ultraviolet B (UVB) light (280-320 nm) is a modality widely used for the therapy of different skin diseases. Initially, broad-band (BB)-UVB light sources were applied in UVB phototherapy, these emit wavelengths throughout the whole spectrum of UVB light (1). In 1980, an action spectrum study in patients with psoriasis was carried out. With use of a monochromator, the action spectrum for the ultraviolet phototherapy of psoriasis was determined for radiation between 254 and 313 nm, and compared with the action spectrum for erythema of the uninvolved adjacent skin. Wavelengths of 254, 280 and 290 nm proved to be erythemogenic, but not therapeutic even at 10 to 50 times the minimal erythema dose (MED). At wavelengths of 300 and 304 nm, complete clearing occurred on daily exposure to doses equal to or less than the MED. In every subject, suberythemogenic exposure doses of 313 nm resulted in complete clearance of the plaques (2). These findings led to the introduction of selective UVB phototherapy and narrow-band (NB)-UVB phototherapy. NB-UVB source emits polychromatic light, but the 311-313 nm wavelength range predominates in its emission spectrum. In a bilateral comparative study, the ability of suberythemogenic doses of NB-UVB versus conventional BB-UVB to remit psoriasis was compared. NB-UVB radiation proved to be superior to BB-UVB for the treatment of psoriasis (3). Since laser light can be selectively directed towards the lesional skin, and all of the energy of a 308 nm excimer laser is emitted within the action spectrum for the phototherapy of psoriasis, our group investigated the therapeutic effect of the 308 nm Xenon chloride (XeCl) excimer laser for psoriasis. This laser emits its total energy at 308 nm and may therefore be regarded as a "super-narrow-band" UVB,light source. The cumulative dose required for the complete clearance of psoriatic plaques was 6 times less with the XeCl laser than with NB-UVB phototherapy (4). The high clinical efficacy of the XeCl laser for psoriasis was later confirmed in other studies (5, 6). The XeCl laser might therefore be regarded as a new and promising form of UVB phototherapy, which seems to be superior to conventional UVB sources in the treatment of psoriasis and vitiligo (7, 8, 9, 10).

UVB light has been shown to modify cutaneous immune responses, a phenomenon that is called photo-immunosuppression (11). Krueger et al. observed that UVB treatment produced a consistent and profound depletion of T lymphocytes from psoriatic epidermis. Dermal

lymphocytes were much less affected (12). As apoptosis is induced by the *in vitro* UVB irradiation of T cells, it has been proposed that UVB light may have immunosuppressive effects in psoriasis through the induction of apoptosis in disease-mediating T cells. Apoptosis, or programmed cell death is a physiological suicide mechanism that preserves homeostasis, in which cell death naturally occurs during normal tissue turnover (13). To characterize the mechanism of T cell depletion, Ozawa et al. applied NB-UVB radiation on peripheral T cells and measured the extent of apoptosis. 100 mJ/cm^2 NB-UVB light resulted in measurable T cell apoptosis by flow cytometry 20 hours later (14). Breuckmann et al. (15) demonstrated delayed apoptosis (within 24-48 hours) on human T cells after BB-UVB irradiation also.

We recently found that XeCl laser is more effective in inducing T cell apoptosis *in vitro* than NB-UVB light, a finding in good agreement with the observed higher clinical efficacy of the laser compared with that of the NB-UVB light (16).

Aims of the study. There is growing evidence that one of the major mechanisms of immunosuppression with UVB light is an apoptosis-inducing effect on T cells infiltrating the skin in inflammatory skin diseases. As different UVB light sources have been used in UVB phototherapy with great differences in clinical efficiency, we examined the apoptosis-inducing capacities of these UVB lamps. Although much work has been performed on the action spectra of UVB radiation for different biological processes, no scientific study appears to have focused on the action spectrum of UVB light for the induction of T cell apoptosis. The aim of our study was to establish such an action spectrum by using different polychromatic UVB light sources.

MATERIALS AND METHODS

UVB light sources. Seven different artificial spectral distribution UVB light were used in the *in vitro* irradiation experiments: an FS20 (Westinghouse, Pittsburgh, PA) lamp without any filter (abbreviation: FS20); an FS20 lamp with tissue culture plate top filter (Corning, London, England) (abbreviation: FS20+Plastic)*; an NB-UVB (Philips TL01, Philips, Eindhoven, the Netherlands) lamp without any filter (abbreviation: TL01); an NB-UVB lamp with 0.055%, 17 mm thick phtalic acid filter in a pyrex tube (abbreviation: TL01+phtalic acid)**; an NB-UVB lamp with a tissue culture plate top filter (Corning, London, England) (abbreviation:

TL01+Plastic)*; a Solar simulator (Oriel ozone free Xe arc lamp, 2000W, Oriel, Stamford, CT) with WG305 filter; 308-nm XeCl excimer laser (Lambda Physik LPX 105 E, Göttingen, Germany) without any filter (abbreviation: XeCl).

*The top of the 24-hole tissue culture plate (Corning, England) was used as a UVB filter.

**The 35 mm diameter original TL01 tube was placed into a 52 mm diameter pyrex tube and the space between the two was filled with 0.055% potassium phthalate which is a UVB filter. The phtalic acid filter has to be refilled after 4 hours of use.

The power of the XeCl laser was metered by an energy detector (Gentec ED-200, Quebec, Canada) and a 100 MHz oscilloscope (WATSU Electronic Co. Ltd., Tokyo, Japan). The output of the non-laser UVB light sources was metered by a calibrated UVB detector (Laser Precision Corp. RT-10l, Utica, CA). The irradiance values and incident doses used for irradiation with the different UVB light sources are shown in Table 1. The spectral irradiances were determined in 1 nm steps with a spectroradiometer (Optronic 742). The spectral distribution of the 7 different UVB light sources is represented in Figure 1. The absolute spectral irradiance values, indicated on the vertical axis, only show values above the noise level of the instrument.

UVB irradiation in vitro. Peripheral blood mononuclear cells (PBMC) were prepared from heparinized venous blood of healthy volunteers by centrifugation over Ficoll-Isopaque gradient. The PBMC were washed twice in phosphate-buffered saline (PBS), and then the irradiation was performed in uncovered tissue culture plates (Corning, London, England) at a density of 10^6 cells/well in 0.3 ml PBS. The preparation of the cells was performed in dark to prevent them from accidental photodamage. In order to prevent a warming side-effect, the PBMC were placed into a 25 °C water bath during irradiation with a solar simulator. After irradiation, the PBMC were washed once in PBS and resuspended in RPMI 1640 (Gibco, Scotland) supplemented with 10% AB+ heat-inactivated human serum, 2 mM L-glutamine and antibiotics. The cells were cultured in a humified 5% CO_2 atmosphere at 37 °C for 24 hours.

T cell apoptosis detection. T cell apoptosis was detected as described earlier (17). Briefly: PBMC were fixed in 2% paraformaldehyde solution for 30 min, washed in PBS and permeabilised in 0.1% saponin in PBS supplemented with 1% fetal bovine serum and 0.02% NaN_3 for 15 min on

ice. The cells were then washed in PBS containing 1% BSA, the supernate was discarded, and each sample was stained with 20 μl Apo2.7-PE monoclonal antibody (mAb) (Immunotech, Paris, France) and 5 μl anti-human CD3-FITC mAb (Dako, Copenhagen, Denmark) in 75 μl PBS for 25 min at room temperature. To exclude false-positive reactions, control samples were stained with isotype-matched anti-human IgG1-PE mAb. After washing, the PBMC were resuspended in 0.5 ml PBS.

Flow cytometry. Sample data were acquired on FACStar and FACSCalibur (Becton Dickinson, Franklin Lakes, NJ) flow cytometers; 10^3 events were collected per sample. The samples were analyzed by using Cell Quest software (Becton Dickinson, Franklin Lakes, NJ) as described earlier (15). Briefly: T cells were selected via anti-CD3 mAb staining, and the Apo2.7 mAb binding of these cells was then quantified by histogram analysis.

Determination of the DNA-weighted efficiency spectra and the median wavelength values. Using the result of the experiments described above, dose-effect curves were created for the induction of T cell apoptosis in the case of each light source. The formula for the biologically effective dose rate (DNA-weighted efficiency spectra) was calculated applying the recommendation of the Commission Internationale d'Éclairage, briefly:

$$BED / t = \Sigma E(\Delta\lambda) * S^{DNA}(\lambda) * \Delta\lambda,$$

where BED/t is the biologically effective dose rate (DNA-weighted efficiency spectra of a UV source), $E(\lambda)$ is the spectral irradiance of the UV source, $S^{DNA}(\lambda)$ is the action spectrum for UV radiation-induced DNA-damage as published by Setlow (18) and $\Delta\lambda$ is the wavelength step from spectroradiometric measurements for the determination of irradiance spectra of the light sources. The areas under these DNA-weighted efficiency spectra were calculated with the use of the rectangle method, briefly: an area under the curve was distributed to many rectangles of 1 nm of width, and the area could has been calculated as the total of the areas of rectangles. The median wavelength was determined as the wavelength value which divides the area under the curve into two equal parts in the case of each light source.

RESULTS

The T cell apoptosis-inducing capacities of different UVB sources

In order to establish an action spectrum of UVB for T cell apoptosis-induction, we used different spectral distributions of UVB light. The BB-UVB or FS20 lamp, the NB-UVB or TL01 lamp, the XeCl laser and the Xe arc solar simulator are well known from the UVB phototherapy of different skin diseases. By using a phtalic acid filter with NB-UVB light and by using the plastic tissue culture plate top as a filter with FS20 and NB-UVB light, we could create 3 more spectral distributions of UVB light. The emission spectra of these 7 UV sources were then determined with a spectroradiometer (Fig. 1). PBMC were irradiated *in vitro* with increasing doses, and T cell apoptosis was measured by means of simultaneous Apo2.7 and anti-CD3 stainings with flow cytometry. Dose-effect curves were then created. All 7 UVB sources induced T cell apoptosis in a dose-dependent manner. Figure 3 represents these dose-effect curves. The dosis necessary to induce apoptosis in 50% of the T cells (AD50) was calculated and listed in Table 2.

TL01 induced apoptosis in 50% of the T cells at a dosis of 300 mJ/cm^2; this result is in good agreement with our previous experimental data (16). The use of a plastic plate top as a filter eliminated most of the short-wavelength UVB radiation. This modification of the spectrum led to a decrease in the T cell apoptosis-induction capacity of NB-UVB and FS20. The filtering effect of phtalic acid was well observable too: a decrease of about 2 orders of magnitude was found at the wavelength 300 nm and phtalic acid eliminated UV radiation almost completely below 300 nm, which resulted in a severe decrease in the apoptosis-inducing capacity of NB-UVB light (AD50: 301 mJ/cm^2 and 553 mJ/cm^2 without and with phtalic acid filter, respectively).

FS20 was the most effective of the 6 non-laser UV sources in inducing T cell apoptosis. It contains considerable amount of UVC light, and shorter UVB wavelengths predominate in its emission spectrum. The elimination of most of this short-wavelength radiation resulted in a decrease in its apoptosis-inducing capacity (AD50: 168 mJ/cm^2 and 210 mJ/cm^2 without and with plastic filter, respectively).

The solar simulator proved to be the least effective in inducing T cell apoptosis. Although it contains some UVC light and a considerable amount of UVB radiation, UVA predominates in its

emission spectrum. The energy density required for the induction of apoptosis in 50% of the T cells was 1912 mJ/cm^2, which is more than 6 times more than the AD50 for NB-UVB light.
The 308 nm XeCl laser is a coherent monochromatic pulse-mode UVB excimer laser with a very high irradiance (approx. 10^9 times more than those of the above mentioned non-laser UVB sources). The XeCl laser induced T cell apoptosis in a dose-dependent manner too, but quantitative induction was much higher than with non-laser UVB sources. The AD50 for the XeCl laser was 95 mJ/cm^2, this laser light therefore induced apoptosis to a higher degree than did any of the non-laser UV sources.

Determination of the wavelength dependence of T cell apoptosis induction

One of the major mechanisms of action of UVB light is the induction of T cell apoptosis. It is well known that the biological effects of UV light are strongly dependent on the wavelength spectrum, though few scientific data exist about the wavelength dependence of T cell apoptosis induction. We decided therefore to construct an action spectrum for T cell apoptosis induction in the UVB range. The above mentioned 6 different polychromatic light sources were used and dose-effect curves were created by performing the irradiation with increasing energies. Data are expressed as the percentages of Apo2.7 and CD3 double-positive cells in relation to all the CD3-positive cells. The energy densities required for the induction of apoptosis in 50% of the T cells were then determined. The apoptosis-inducing capacities of each UV light source were calculated by reciprocating the AD50 values (Table 2). DNA damage seems to be the major mechanism in the background of UVB radiation-induced T cell apoptosis (15), thus the emission spectra of the polychromatic UV sources were weighted by the action spectrum of UV radiation-induced DNA damage as described above. These DNA-weighted efficiency spectra of the UV sources used are presented in Figure 3. The median wavelength values of the areas under the curves were calculated and presented in Table 2. For constructing the action spectrum for T cell apoptosis induction at polychromatic UVB sources the modification of the method applied in (19) was used as follows. The median wavelength value of each UV source has been associated with its apoptosis-induction capacity (1/AD50 value) obtained from the dose-effect curves and a linear regression curve was calculated. Figure 4 depicts the wavelength dependence of T cell apoptosis induction in the range 290 to 311 nm. This regression curve revealed a consistent

decrease from shorter to longer wavelengths, e.g. irradiation at 290 nm is 3 times more effective in T cell apoptosis induction than that of 311 nm. The real apoptosis inducing capacity of the XeCl laser is almost 4 times higher than that calculated on the basis of thisspectrum.

DISCUSSION

UV phototherapy is widely applied to treat different dermatoses. The prototypic skin disease showing a favorable response to UV phototherapy is psoriasis vulgaris. There is growing evidence that the efficacy of UVA and UVB phototherapy may not simply be attributed to antiproliferative effects, but most likely involves immunomodulatory consequences (20). One of the major mechanisms of action of UVB light in the treatment of inflammatory dermatoses seems to be a cytotoxic effect on the infiltrating T cells, where the mechanism of cell death is most probably apoptosis. Earlier, psoriatic plaques in 23 patients were treated daily with NB-UVB or BB-UVB light in a bilateral comparison study. NB-UVB cleared the psoriatic plaques more effectively than did BB-UVB light. On the other hand, NB-UVB light has been found to be a more potent inductor of T cell apoptosis *ex vivo* than BB-UVB light; therefore, the T cell apoptosis-inducing capacity of a UVB light source can be paralleled by its clinical efficacy. In our present study, the *in vitro* T cell apoptosis-inducing capacities of widely used BB-, NB-UVB sources and the XeCl UVB laser were determined and compared with each other. The XeCl laser was the strongest apoptosis-inducer, while BB-UVB (FS20) induced T cell apoptosis more efficiently than did NB-UVB (TL01). In clinical studies, the XeCl laser also seems to be the most efficient antipsoriatic UVB source, suggesting that the more effective induction of T cell apoptosis may be responsible for the greater clinical efficacy of the XeCl laser as compared with non-laser UVB light. Clinically, however, NB-UVB is a more potent antipsoriatic light source than BB-UVB. The main reason for the difference between the *in vitro* and the clinical data might be the absorbance of most of the radiation shorter than 300 nm by the epidermis. Thus, the short-wavelength UVB range that is emitted by BB-UVB induces apoptosis efficiently *in vitro*, but cannot penetrate into the dermis.

Although much scientific work has been carried out on the biological effects of UVB light, little is known about the role of optical parameters of radiation in the effect of UVB radiation on biological processes. The DNA-damaging effect of UVB is mediated through cyclobutane

pyrimidine dimer and (6-4) photoproduct formation. Matsunaga et al. determined the action spectra for the induction of thymine dimers and (6-4) photoproducts in DNA by nearly monochromatic UV light ranging from 150 nm to 365 nm. The most efficient wavelength for the formation of UV light-induced DNA damage proved to be 260 nm, corresponding to the absorption spectrum of DNA (21). Urocanic acid is a major UV chromophore in the upper layers of the skin where it is found predominantly as the *trans* isomer. UVB irradiation induces photoisomerisation of *trans* urocanic acid to *cis* isomer. *Cis* urocanic acid is suggested to be a mediator of UVB light-induced immunosuppression, because its administration can mimic many of the effects of UVB light on the skin (22). Gibbs et al. investigated the wavelength dependence for *trans*-to-*cis* photoisomerisation *in vitro* over the spectral range 270-340 nm, and found that the resulting action spectra had maximal effectiveness at 300-315 nm (23). The mixed epidermal cell lymphocyte reaction (MECLR) and the mixed lymphocyte reaction (MLR) have been commonly used to study the immunosuppressive effects of UVB radiation. The alloactivating capacity in MLR and MECLR experiments is decreased by exposure to UVB light. Using monochromators, Hurks et al. irradiated PBMC with nearly monochromatic UVB light at 254, 297, 302 and 312 nm, measured the decreases in the MLR and MECLR responses, and hence determined the action spectra for the MLR and MECLR from 254 nm to 312 nm. Both the MLR and MECLR action spectra displayed a maximum at 254 nm and a relative sensitivity at 312 nm. The action spectra were strikingly similar to those for the induction of pyrimidine dimers and 6-4 photoproducts (24).

The present study also describes the determination of the wavelength dependence of UVB radiation-induced T cell apoptosis. As 95% of the UVC radiation is scattered, reflected and/or absorbed in the upper thin (about 25-30 μm) part of the epidermis (25), and UVB was found to be superior in biological efficacy than UVA, we restricted the range of our interest to the UVB spectrum. The spectral sensitivity for various biological UV effects, like killing efficiency of simple organisms, production of erythema, DNA damage, skin cancer induction, immunosuppression has been determined mostly with monochromatic light [21, 23, 24]. However, in this case, the determination of the precise sensitivity value with a monochromatic source is somewhat obscure due to the difficulty in determining the accurate light dose (e.g. as monochromatic light created by monochromators is of variable and low intensity, the irradiation procedure would take hours to induce T cell apoptosis). This fact appears in the literature as the

discrepancy between sensitivity curves measured with a monochromatic source and with polychromatic radiation observed in normal human skin fibroblasts (26). A precise and comprehensive action spectrum determination has been performed earlier [27, 28] for Utrecht-Philadelphia skin cancer induction on hairless mice using 14 different polychromatic UV sources. In the 250-400 nm wavelength range the approximation by a Langrange polynomial fourth order resulted in a satisfactory fitting of the calculated and the measured data.

By using different filters with the above-mentioned UV light sources, we could create more spectral distribution UV light with higher intensity. Then the efficacy of different spectral regions of UVB light to induce T cell apoptosis was determined by the use of these polychromatic UV light sources. This experimental method is accepted and proved to be successful in photodermatology completed by a polynomial approximation (28). The emission spectra of sources used were weighted by the action spectrum of UV radiation-induced DNA damage as published by Setlow. The T cell apoptosis-induction capacities of all these lamps were determined by the method described earlier. Dose-effect curves were created and the apoptosis-induction efficacy (1/AD50) of the UVB sources were calculated by reciprocating the dose values which induced apoptosis in 50% of the T cells in the case of each lamp. The wavelength dependence of UVB light to induce T cell apoptosis was determined by associating the 1/AD50 value with the median wavelength of each light source. The regression curve of this "action spectrum" demonstrates a continuous decrease from 290 nm to 311 nm. The decreasing trend of this spectrum is similar to those observed earlier, e.g. the erythema action spectrum, the action spectrum for thymine dimer and 6-4 photoproduct formation and the action spectra for suppression of the MLR and MECLR responses. However, these action spectra (erythema, thymine dimer) show several orders of magnitude difference in efficiency between 290-311 nm while the "action spectrum" reported here show differences far less than this between these wavelengths. The explanation for the similarities might be that all of these processes are predominantly mediated by UV light-induced DNA damage. The apoptosis-inducing efficacy of the XeCl laser proved to be much higher than those of all of the non-laser sources. The high efficacy of the UVB laser was observed in clinical studies too, this super-narrow-band light is therefore used more and more widely in phototherapy of different skin diseases. The explanation of its very high efficacy is still unknown, but we hypothesize that mainly its high irradiance is responsible. Hurks et al. examined the influence of irradiance on the MECLR both *in vitro* and *in*

vivo using BB-UV lamp with variable UV intensities. They found that the irradiation of epidermal cells with high irradiance impaired the alloactivating capacity more than did irradiation with low irradiance. They concluded that UVB radiation-induced suppression of MECLR was critically dependent on irradiance (29). Our results indicated that the irradiance may influence the effects of UVB radiation: higher UVB irradiance can result in a higher level of UVB induced immunosuppression.

As the extent of T cell apoptosis induction and the penetration of UVB light into the dermis is wavelength-dependent, the wavelength spectrum of optimal dermato-phototherapy might depend on the thickness of the skin lesion. We consider that longer wavelength UVB phototherapy would be ideal for the treatment of thick psoriatic plaques, while short-wavelength UVB radiation would be optimal for the treatment of thin skin lesions.

NB-UVB contains considerable amount of short-wavelength UVB light. When phtalic acid filter was used, almost all of the radiation shorter than 300 nm could be eliminated, however, this short-wavelength spectrum was very effective in our *in vitro* study. Earlier measurements disclosed that this short-wavelength-UV light is almost completely absorbed in the upper part of the epidermis (30), and therefore cannot induce directly T cell apoptosis *in vivo*. Thus, the use of phtalic acid-filtered UVB light in skin phototherapy might have advantages over traditional UVB phototherapy by eliminating the erythema-inducing, but therapeutically not efficient short-wavelength- UV radiation. However, this hypothesis remains to be proven by *in vivo* clinical studies.

Although T cell apoptosis induction seems to be the major mechanism of UVB light-induced immunosuppression, the results of this study cannot be directly adapted to clinical application. We are of the opinion that the spectral distribution of a UVB light source influences the clinical results by affecting the apoptosis-induction capacity and the absorbance and transmittance of the light in the epidermis. Light of shorter wavelengths seems to induce T cell apoptosis more efficiently; while that of longer wavelengths penetrates better into the dermis. The determination of the wavelength dependence of UVB-induced T cell apoptosismight be a small step to the improvement of UVB phototherapy.

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FIGURES

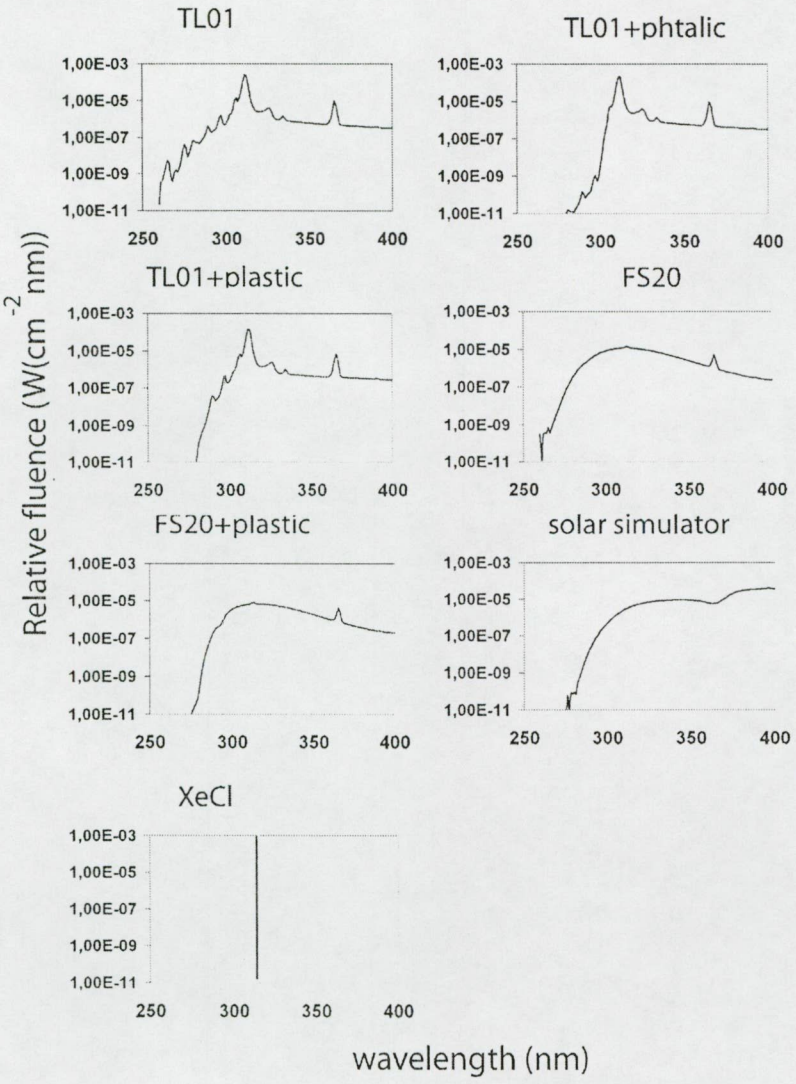

Figure 1. The spectral distribution of the 7 different UVB light sources determined at 1 nm steps with a spectroradiometer. The absolute irradiance values, indicated on the vertical axis, show values above the noise level of the instrument.

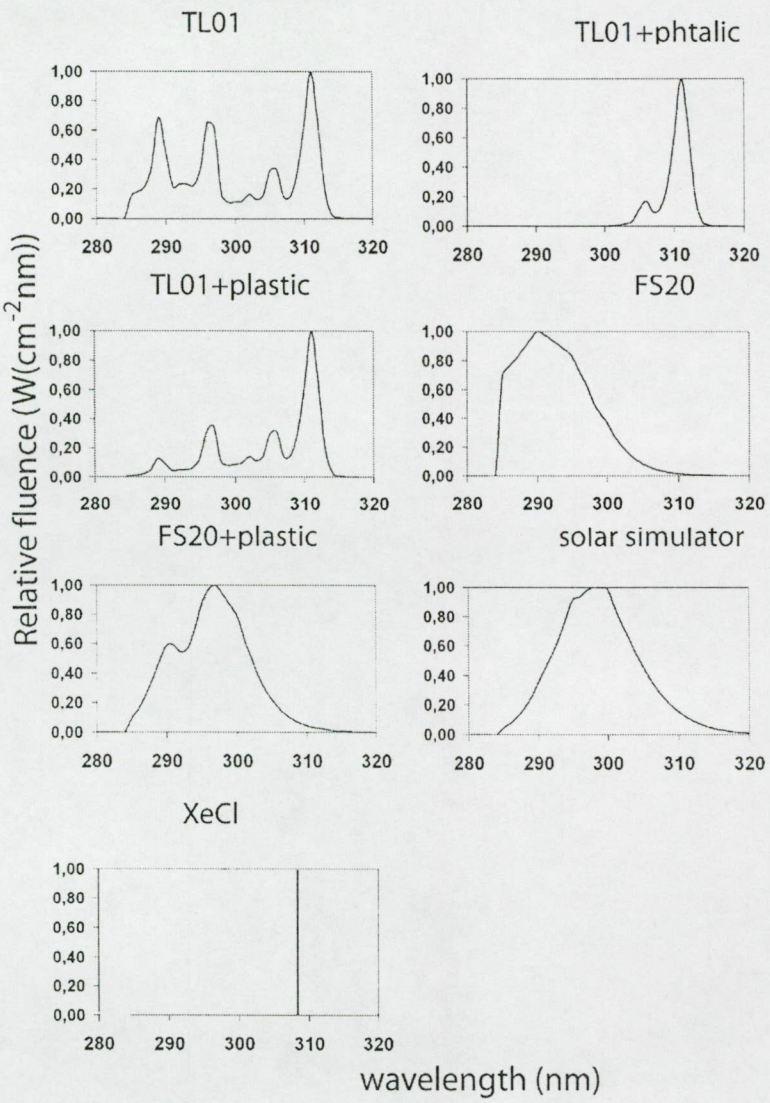

Figure 2. The emission spectra of the polychromatic UV sources were weighted by the action spectrum of UV induced DNA damage resulting in efficiency spectrum (calculated as described in Materials and Methods).

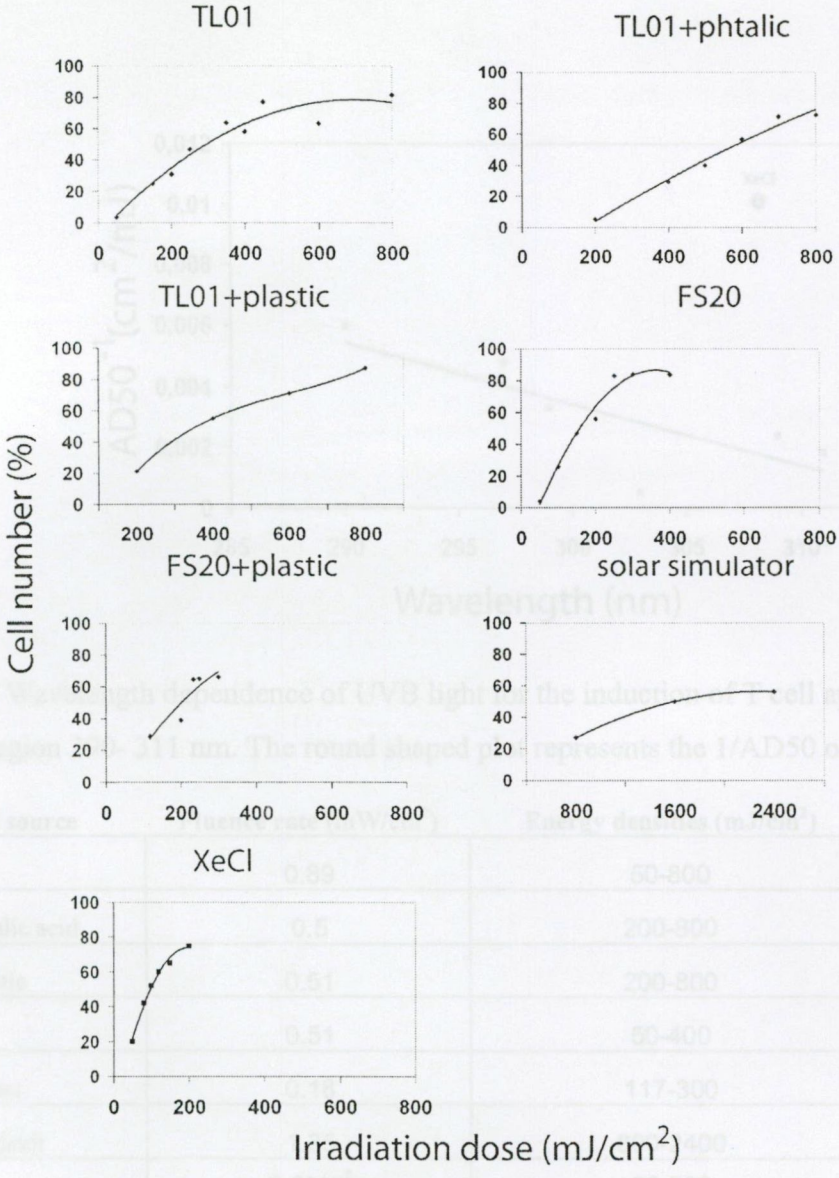

Figure 3. Dose-dependent induction of T cell apoptosis after irradiation with various polychromatic UVB light sources. Data are expressed as the percentage of Apo2.7 and CD3 double positive cells in relation to the all CD3 positive cells. Third order polynomial regression line was fitted to the individual data.

UVB source	wavelength median (nm)	AD50 (mJ/cm2)	1/AD50 (cm2/mJ)
TL01	299	301	0,0033
TL01+phtalic acid	311	553	0,0018
TL01+plastic	309	420	0,0024
FS20	290	168	0,0060
FS20+plastic	297	210	0,0048
Solar simulator	299	1912	0,0005
XeCl laser	308	95	0,0105

Table 2. The median values of the spectral distribution of UVB sources corrected by the action spectrum of UV induced DNA damage (calculated as described in Materials and Methods). The AD50 values or the doses necessary to induce apoptosis in 50% of the T cells were calculated from the dose-effect curves of each lamp. The apoptosis inducing capacities (1/AD50) of each UV light sources were calculated by reciprocating the AD50 values.

Publication II.

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Xenon chloride ultraviolet B laser is more effective in treating psoriasis and in inducing T cell apoptosis than narrow-band ultraviolet B

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Abstract

Earlier we reported that a 308-nm xenon chloride (XeCl) UVB laser is highly effective for treating psoriasis. As ultraviolet B light seems to cause T cell apoptosis, in the present study we studied the ability of the XeCl laser to induce T-cell apoptosis in vitro, and then compared the apoptosis-inducing capacities of narrow-band UVB (NB-UVB) light and the XeCl laser. The role of laser impulse frequency and intensity in the therapeutical and apoptosis-inducing efficacy of XeCl laser was also investigated. Both XeCl laser and NB-UVB induced T cell apoptosis, but quantitative induction was greater with XeCl laser. Changes in the frequency and intensity of impulses of XeCl laser did not influence its therapeutic and T cell apoptosis-inducing efficacy. These results suggest that the more effective induction of T cell apoptosis can be responsible for the greater clinical efficacy of XeCl laser compared to NB-UVB. Additionally, the optical properties of the XeCl laser (a monochromatic, coherent, pulse-mode laser; easier precise dosimetry, there are no 'contaminating' wavelengths) can make this laser light an ideal tool for studies of the mode of action of UVB light.

Keywords: Ultraviolet B; Apoptosis; T cells; Psoriasis

1. Introduction

Psoriasis vulgaris is a chronic inflammatory skin disease that affects 2–3% of the population. Ultraviolet B (UVB) light (290–320 nm) is a widely used therapeutic modality for the disease. Initially, broad-band (BB)-UVB light sources were applied which emit wavelengths throughout the whole spectrum of UVB light. An action spectrum study in patients with psoriasis established that at wavelengths from 304 to 313 nm suberythemogenic exposure doses resulted in complete clearing, while wavelengths from 290 to 300 nm produced the sunburn reaction, but had no therapeutic benefit [1]. These findings led to the introduction of the narrow-band (NB)-UVB light source in the therapy; this also emits polychromatic light, but the 311–313 nm wavelength range predominates in its emission spectrum. In a bilateral comparative study, NB-UVB light proved superior to BB-UVB for the treatment of psoriasis [2].

Xenon chloride (XeCl) UVB laser treatment is a new and promising form of therapy for psoriasis vulgaris [3], because all of its energy is emitted within the action spectrum for the phototherapy of psoriasis (Fig. 1). This laser emits its total energy at 308 nm and may therefore be regarded as a 'super narrow band' UVB light source. We demonstrated earlier that the cumulative dose required for the complete clearance of psoriatic plaques is six times less with the XeCl laser than with NB-UVB therapy [3]. The high clinical efficacy of the XeCl laser for psoriasis was later confirmed by other studies [4,5]. The clinical efficacy of the XeCl laser in psoriasis is therefore well documented, but the mechanism of its high efficacy has not been investigated so far.

There is considerable evidence, that psoriasis vulgaris is mediated by activated T-lymphocytes infiltrating the epidermis and the dermo-epidermal interface [6]. The major mechanism of action of UVB light in the treatment of inflammatory dermatoses is the cytotoxic effect on the

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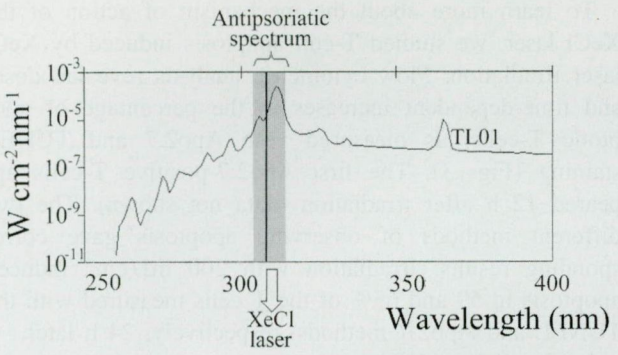

Fig. 1. Emission spectra of NB-UVB (TL01) and 308 nm XeCl laser. The spectrum of Philips TL01 UVB source was obtained from Modos et al. [12]. The gray area indicates the most effective wavelengths for the phototherapy of psoriasis as measured by Parrish et al. [1].

infiltrating T-cells, where the mechanism of cell death is most probably apoptosis. NB-UVB light has been found to be a more potent inductor of T-cell apoptosis than BB-UVB light, therefore, the T-cell apoptosis-inducing capacity of a UVB light source can be paralleled by its clinical efficacy [7].

In the present study, we investigated whether changes of the light intensity and impulse frequency of the XeCl laser influence its therapeutic efficacy. As UVB light seems to cause T-cell apoptosis, we studied the ability of the XeCl laser to induce T-cell apoptosis in vitro, and then compared the apoptosis-inducing capacities of NB-UVB light and the XeCl laser.

2. Patients and methods

2.1. Patients

A total of 48 plaques of 21 patients with chronic plaque type psoriasis were treated with XeCl laser phototherapy. Informed consent was obtained before the start of the study. Upon entry to the study, the patients had not been treated with systemic antipsoriatic medication for a minimum of 4 weeks. For each patient, a minimum erythema dose (MED) was established in uninvolved, unexposed gluteal skin. XeCl laser therapy was given three times weekly until the treated plaques had cleared completely. The initial dose was 0.6 MED, which was increased by 20% on each subsequent treatment. A 308-nm XeCl excimer laser (Lambda Physik LPX 105 E, Göttingen, Germany) was used: its output consisted of a train of short pulses (15 ns) at 5.5 mJ/cm^2 per pulse (the size of the light spot is 3×3 cm). In two groups of patients, XeCl laser phototherapy was performed with different impulse intensities (0.06 and 20 mJ/cm^2) or impulse frequencies (1 and 20 Hz), and the cumulative doses and the number of treatments up to complete clearance were determined. In two groups of patients, symmetrical psoriatic plaques were irradiated with the XeCl laser with an impulse frequency of 1 or 20 Hz, and local psoriasis severity index (LPSI) [8] scores were determined for each plaque following each treatment.

2.2. UVB irradiation in vitro

Peripheral blood mononuclear cells (PBMC) were prepared from venous blood of healthy volunteers by Ficoll sedimentation. The irradiation was performed with the 308 nm XeCl laser or a 311-nm NB-UVB lamp (Philips TL-01, The Netherlands) in uncovered tissue culture plates (10^6 cells/well) in phosphate-buffered saline (PBS). For this study, laser radiation was delivered at 1–40 impulses/s. The power of the XeCl laser was metered by an energy detector (Gentec ED-200, Quebec, Canada) and a 100-MHz oscilloscope (Watsu Electronic Co. Ltd., Japan) The output of the NB-UVB light was metered by a calibrated UVB detector (Laser Precision Corp. RT-10 CAL, USA). After irradiation, the PBMC were washed once and resuspended in RPMI 1640 (Gibco, Scotland) supplemented with 10% AB+ heat-inactivated human serum, 2 mM L-glutamine and antibiotics. The cells were cultured at 37 °C in 5% CO_2 for different time intervals.

2.3. Detection of apoptotic cells

2.3.1. TUNEL labeling

The TUNEL (TdT-mediated dUTP-FITC nick end labeling) reaction was performed according to the manufacturer's instruction (Boehringer Mannheim, Switzerland). Briefly: PBMC were fixed in 4% paraformaldehyde solution for 30 min, washed twice in PBS, permeabilised in 0.1% Triton X-100 in 0.1% sodium citrate for 2 min on ice, washed twice in PBS containing 1% BSA, the cells were then labeled with the TUNEL reaction mixture at 37 °C for 60 min and anti-CD3-PE monoclonal antibody (mAb) (Dako, Denmark) on ice for 15 min. After washing in PBS, the cells were resuspended in 0.5 ml PBS.

2.3.2. Apo2.7 labeling

PBMC were fixed in 2% paraformaldehyde solution for 30 min, washed in PBS and permeabilised in 0.1% saponin in PBS supplemented with 1% fetal bovine serum and 0.02% NaN_3 for 15 min on ice. The cells were then washed in PBS containing 1% BSA, the supernatant was discarded, and each sample was stained with 20 μl Apo2.7-PE mAb (Immunotech, France) and 5 μl anti-human CD3-FITC mAb (Dako, Denmark) in 75 μl PBS for 25 min at room temperature. To exclude false positive reactions, control samples were stained with isotype-matched anti-human IgG1-PE mAb. After washing, cells were resuspended in 0.5 ml PBS.

2.3.3. Flow cytometry

Sample data were acquired on FACStar and FAC-

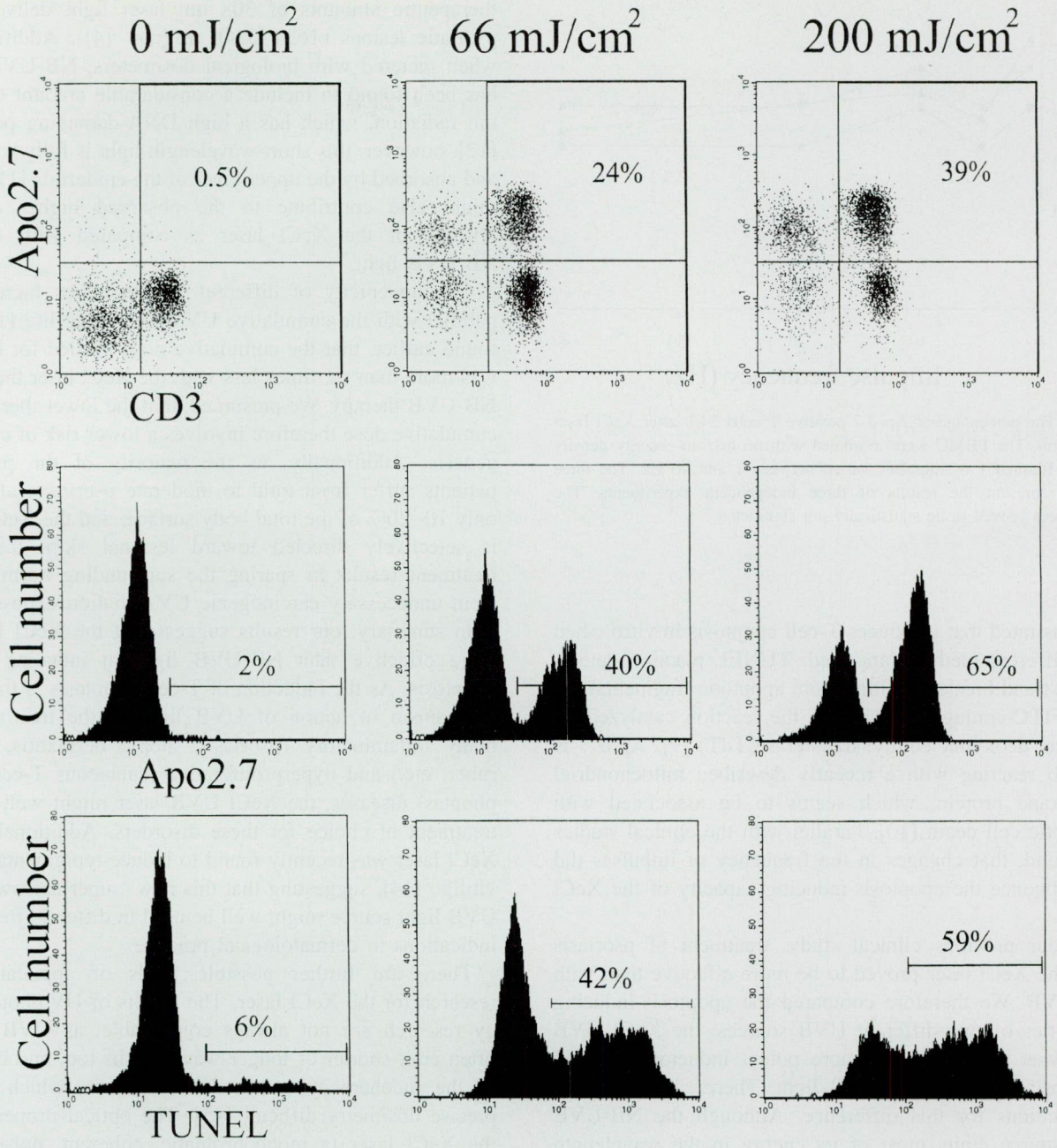

Fig. 3. Flow cytometric analysis of T-cell apoptosis. PBMC were irradiated with increasing amounts of energy from the XeCl laser, and apoptosis was assessed 24 h after irradiation. The left panels are untreated controls. The top panels show simultaneous CD3 and Apo2.7 stainings; the upper right quadrant indicating apoptotic T-cells. The middle and bottom histograms show only CD3+ T cells, as identified by FITC-CD3 or PE-CD3 staining. The abscissa represents the extent of apoptosis, measured with the Apo2.7 or TUNEL method.

impulse frequencies. We did not find significant differences in either of the investigated parameters.

For most of the plaques, the 3×3-cm light spot of the XeCl laser is sufficient for treatment, however we are seeking possibilities to extend the area being irradiated at a given time. One potential solution would be to mount the XeCl laser with a real-time scanner [4]. The scanner would perform a total body scan to recognize psoriatic plaques and would automatically direct the laser light toward the involved areas. However, this technique would result in the same plaque receiving irradiation at a reduced impulse frequency. Another possibility for total body treatment is if the laser light is scattered to increase the area of irradiation. In this case, the light intensity of the laser impulses would be reduced. Our present results indicate that the introduction of a scanning system or laser light scattering would not lower the efficacy of XeCl treatment.

As UVB acts through the induction of apoptosis on different cell types, we set out to learn more about the cellular mechanism of action of the XeCl laser, and

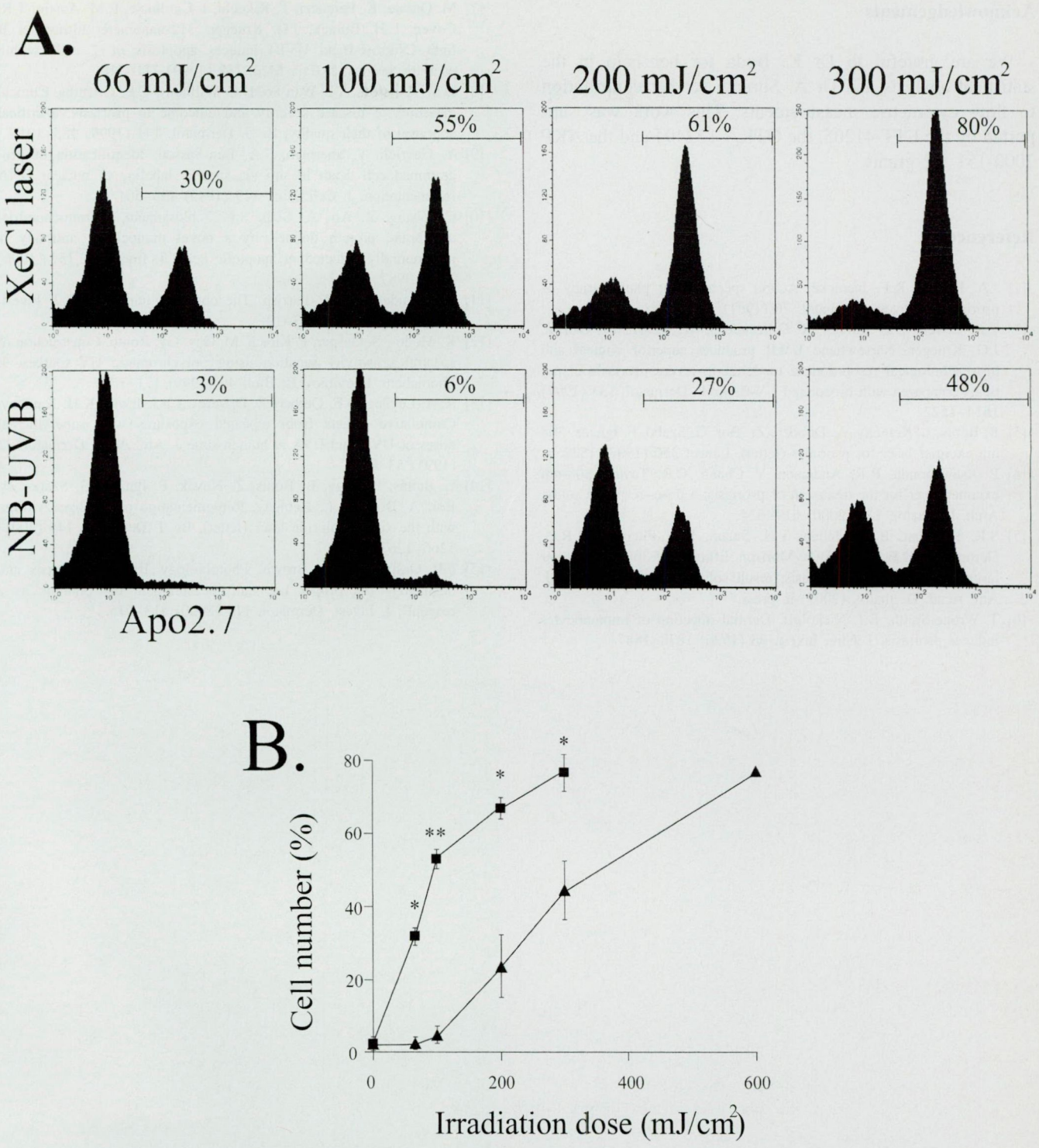

Fig. 5. Comparison of the T-cell apoptosis-inducing capacities of NB-UVB light and the XeCl laser. PBMC were irradiated with increasing amounts of NB-UVB light and XeCl laser light. Apoptosis was measured with Apo2.7 staining 24 h after irradiation. All of the histograms show only CD3+ T cells. (A) The extent of apoptotic T cells are represented on the abscissa following NB-UVB (upper panels) or XeCl laser (lower panels) irradiation with the same energy densities. (B) Summary of three independent experiments. The mean percentage of Apo2.7-positive cells is represented after irradiation with increasing amounts of NB-UVB light (triangles) or XeCl UVB laser light (squares). Irradiation with 600 mJ/cm^2 XeCl laser induced necrosis of T cells, therefore apoptotic changes could not be detected. The paired t-test was performed. P values: * $P<0.005$, ** $P<0.0005$.

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Repigmentation of localized vitiligo with the xenon chloride laser

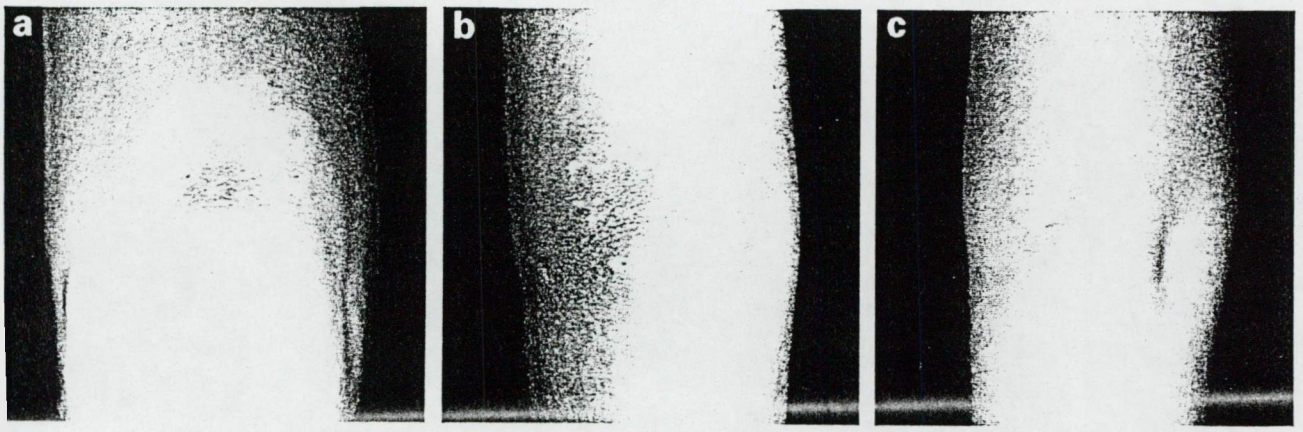
SIR, Vitiligo is a common idiopathic acquired disorder of unknown cause, which produces disfiguring white patches of depigmentation. A large variety of therapeutic agents has been tried for its treatment, but no definitive cure is yet available. Topical and systemic steroids have been reported to display variable efficacy in the treatment of vitiligo.[1] The most widely used procedure has been phototherapy alone or in combination with topical corticosteroids or pseudocatalase.[1–3] One of the most frequently used forms of phototherapy is topical psoralen plus ultraviolet (UV) A (PUVA). However, there is concern over its long-term effects, especially in children, with regard to the increased risks of carcinogenesis and premature ageing of the skin. L-Phenylalanine plus UVA treatment has also been reported to lead to a good therapeutic effect in children with extensive vitiligo, but this result was not confirmed by others.[4] Narrow-band UVB (NB–UVB) phototherapy has recently been found to be highly effective and well tolerated in both adults and children.[5–7] NB–UVB therapy has been claimed to have fewer adverse effects compared with PUVA therapy. Although insufficient human data are available, it has been calculated that long-term NB–UVB therapy may involve a lower risk of skin cancer than that of PUVA therapy.[8]

We recently found that the 308-nm XeCl excimer laser is more effective than NB–UVB (311–313 nm) for the treatment of psoriasis, suggesting that this UVB laser might offer advantages over NB–UVB.[9] The purpose of this study was to evaluate the effectiveness of 308-nm XeCl laser phototherapy in vitiligo.

A 24-year-old woman (skin type III) in otherwise excellent general health presented with vitiligous patches of several years' duration on the elbows. There had been no spontaneous repigmentation. She had been treated unsuccessfully with topical steroids. Her family history was negative for pigmentary disorders.

We used a 308-nm XeCl excimer laser (Lambda Physics LPX 105 E). The energy of each light impulse was 5·5 mJ cm^{-2}, with a duration of 15 ns; the diameter of the light spot was 3 cm. Irradiation was started at nine impulses, at a rate of 20 impulses s^{-1}. The vitiligous patches on the elbows were treated three times weekly for 5 weeks initially, then twice weekly, never on consecutive days. The initial dose was 49·5 mJ cm^{-2}, which was increased by 49·5 mJ cm^{-2} at each session. The cumulative dose of irradiation was 70·8 J cm^{-2}. During the treatment the eyes were protected with UV-blocking goggles. Photographs of the elbows were taken 1 week before the commencement of laser therapy, after 6 months of treatment, and 3 months following termination of the laser therapy.

Before the treatment, approximately 3-cm diameter completely white macules were seen on both elbows (Fig. 1a).



igure 1. The elbow is shown (a) prior to treatment (b) after 6 months of treatment, and (c) 3 months after termination of the therapy.

Repigmentation was first observed 3 months after the start of the therapy, with pigmented macules of 1–3 mm in diameter. During the therapy continuous repigmentation was subsequently observed, localized to the hair follicles. This process led to nearly complete repigmentation of the vitiligous areas within 6 months (Fig. 1b). Three months after the termination of the laser therapy, the repigmentation was stable (Fig. 1c). No phototoxic or photoallergic reactions, perilesional hyperpigmentation or other side-effects were observed. Our patient was fully satisfied with the cosmetic result. One year after stopping the laser therapy the repigmentation is still stable, although relapse is still possible, as with other phototherapies.

In the present study, we found that an XeCl UVB laser was highly effective in one patient for the treatment of vitiligo. As only the vitiligous areas were treated by UVB, there is little risk of carcinogenesis or other UVB side-effects occurring in the surrounding skin. Additionally, the absence of perilesional hyperpigmentation results in a better cosmetic appearance as compared with that attained with other forms of phototherapy. We suggest therefore that the 308-nm UVB excimer laser may be a valuable and safe option for vitiligo therapy.

The mechanism of UV-induced repigmentation is unknown. It is likely that, similar to NB–UVB, the 308-nm UVB laser exerts immunomodulatory effects and may also stimulate the melanocytic reserves in the hair sheaths, as repigmentation occurs in a perifollicular pattern. Further investigations should be conducted to elucidate the exact mechanisms of action.

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Pulsed dye laser treatment for inflammatory linear verrucous epidermal naevus

SIR, Inflammatory linear verrucous epidermal naevus (ILVEN) is the term used to describe epidermal naevi in which inflammatory changes are a prominent clinical feature.[1] They are often not present at birth and appear during the first 5 years of life, occasionally later. Most appear during the first 6 months and usually persist, although they may occasionally resolve spontaneously. Various treatments have been tried, none being entirely satisfactory. The lesions are pruritic and often cosmetically unsightly. We report three patients with ILVEN, all of whom received successful treatment with the pulsed dye laser (Candela SPTL IB, 585 nm).

Patient 1. An 8-year-old boy had extensive ILVEN lesions, present since birth, on the right cheek, palm of the right hand (including palmar aspects of the index, middle and ring fingers) and the right buttock extending down the posterior aspect of the leg to the medial side of the great toe (Fig. 1a). Over time, these lesions had extended and become more scaly. He had a history of eczema but, apart from his skin problems, was otherwise well. Previous treatment included emollients and topical steroids of moderate potency. This had caused some reduction in the erythema on the cheek but no change in the other lesions.

At 3 years of age laser treatment was commenced. Treatment of an initial test area on the right thigh, using the pulsed dye laser at 585 nm and 7 J cm^{-2} with a 7-mm spot size, showed a good response. A more extensive treatment was then performed to the right thigh and buttock (585 nm, pulse width 0·45 ms, 6·5–7·5 J cm^{-2}, 7-mm spot size) under general anaesthesia. Following this treatment the lesion was smoother and less erythematous, and some areas had disappeared totally. A further four treatments were performed to the buttock and leg using the same energy dose and spaced 6 months–1 year apart. The cheek was treated on three occasions (585 nm, pulse width 0·45 ms, 6·25–6·5 J cm^{-2}, 7-mm spot size). The leg showed the best response, with resolution of the scaling and erythema, and flattening of the lesion (Fig. 1b). The face showed some, but less marked, improvement. These results were maintained over a follow-up period of 2 years.

Patient 2. A 7-year-old boy had had two lesions of ILVEN from birth, one on the palm of the left hand extending to the three middle fingers and the other on the medial sole of the left foot. He also suffered from cerebral palsy and

Publication IV.

UVB irradiation-induced apoptosis increased in lymphocytes of Huntington's disease patients

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by CAG repeat expansion in the IT-15 gene coding for huntingtin. The mechanism of neuronal degeneration induced by the mutant huntingtin is not known. Apoptosis may play a role in it. Huntingtin is widely expressed in the cells, so abnormalities can be expected also in non-neural tissue. We examined the susceptibility of lymphocytes from HD patients, asymptomatic carriers and normal individuals to UVB irradiation-induced apoptosis. Lymphocytes from eight HD patients and two asymptomatic carriers showed increased apoptotic cell death compared to controls. Our results suggests that sensitivity of HD cells to induced apoptosis is not restricted to neurons. *NeuroReport* 12:1653–1656 © 2001 Lippincott Williams & Wilkins.

Key words: Apoptosis; Huntington's disease; Lymphocyte; UVB irradiation

INTRODUCTION

Huntington's disease (HD) is a neurodegenerative disorder with autosomal dominant inheritance. The main symptoms are choreiform involuntary movements and changes in personality culminating in dementia [1]. The characteristic neuropathological feature of HD is the selective loss of medium-size spiny neurons in the striatum and the loss of large cortical neurons in layer VI [2]. The genetic defect is a CAG repeat expansion in the IT-15 gene coding for a protein with unknown function, named huntingtin [3]. The mechanism of cell death induced by the toxic gain of function of mutant huntingtin is not known, but there is increasing evidence that apoptosis may play an important role in neurodegeneration. Apoptotic neurons were detected by TUNEL staining in the neostriatum of HD patients [4,5], and activated caspase 8 was identified in neuronal intranuclear inclusions in HD brains [6]. This suggests that the inclusions composed of polyglutamine repeats of huntingtin can induce apoptosis by catalysing caspase activation. Mutant huntingtin with polyglutamine stretch is cleaved by proapoptotic enzyme caspase 3 (apopain) [7,8]. Huntingtin is widely expressed throughout the CNS as well as in non-neural tissues, so functional abnormalities can be expected also outside of the brain [9]. We wanted to know whether non-neural tissue shows increased sensitivity to apoptosis. The susceptibility of lymphocytes from HD patients, asymptomatic carriers to UVB irradiation-induced apoptosis was examined and compared to normal controls.

MATERIALS AND METHODS

Patients: Peripheral blood mononuclear cells (PBMC) were prepared from venous blood of eight HD patients, two asymptomatic carriers and 10 healthy controls by FICOLL sedimentation. HD patients were 49.6 ± 15.7 (mean $\pm$ s.d.) years old, asymptomatic carriers were 37 and 45 years old, control individuals were 45 ± 10.8 years old. The number of CAG repeat ranged from 44 to 70 in HD patients. The asymptomatic carriers had 43 and 45 CAG repeats. All of the patients, asymptomatic carriers and controls voluntary participated in the study. The guidlines for the molecular genetics predictive testing in HD was followed [10].

UVB irradiation: For induction of apoptosis PBMC were irradiated by XeCl 308 nm UVB laser (Lambda Physik LPX 105E) in uncovered tissue culture plates in PBS. Laser radiation was delivered at 20 Hz, and radiation doses were 70–200–300 mJ. After irradiation the PBMC were washed once and suspended in RPMI 1640 (Gibco, Scotland) supplemented with 10% AB+ heat inactivated human serum, 2 mM L-glutamine and antibiotics. The cells were cultured at 37°C in 5% CO_2 for 20 h.

Immunofluorescence assay for flow cytometry: PBMC were fixed in 2% paraformaldehyde for 30 min, washed in PBS and permeabilised in 0.1% saponin in PBS supplemented with 1% fetal bovine serum and 0.02% NaN_3 for 15 min on ice. The cells were washed in PBS containing 1% BSA, the supernate was discarded and each sample was stained with 20 µl APO2.7-PE mAb (Immunotech, Marseille, France) and 5 µl anti-human CD3-FITC mAb (Dako, Glostrup, Denmark) in 75 µl PBS for 25 min at room temperature. The APO2.7-PE mAb reacts to a 38 kDa mitochondrial membrane protein exposed on cells undergoing apoptosis [11]. To exclude false positive reactions control samples were stained with isotype-matched anti-mouse mAb. After washing cells were resuspended in 0.5 ml PBS.

Flow cytometry: Sample data were aquired on FACStar and FACSCalibur (Beckton Dickinson, USA) flow cytometer. Samples were analysed using CellQuest software (Beckton, Dickinson, USA). The percentage of APO2.7-PE positive CD3+ lymphocytes were determined.

Statistical analysis: One-way ANOVA was followed by the Fisher's LPSD test to determine significant differences between groups. $p < 0.05$ was considered statistically significant.

RESULTS

Lymphocytes from normal control individuals cultured for 20 h without UVB irradiation underwent apoptosis to a minimal degree. Lymphocytes from HD patients cultured in the same condition showed no increased apoptosis (3.43%).

UVB irradiation at an intensity of 70 mJ induced apoptotic changes in one-fifth of lymphocytes from normal individuals cultured exactly in the same condition. Higher intensities of irradiation linearly increased the percentage of lymphocytes labelled with antibody recognizing apoptotic mitochondrial membrane protein. However, the same intensities of UVB irradiation of cultured lymphocytes from HD patients rendered more lymphocytes to undergo apoptotic process than in control groups. Each intensity of UVB irradiation applied induced apoptosis in significantly higher percentage of lymphocytes from HD patients than from normal controls ($p < 0.0171$, $p < 0.0099$, $p < 0.0126$; Fig. 1).

The reactivity seems to be uniform. All of the samples from HD patients exposed to irradiation showed higher proportion of apoptotic cells than any of the controls (Fig. 2). Furthermore the same rate of induced apoptosis was noted in the lymphocytes of two asymptomatic carriers of HD gene. The enchancement of the apoptotic reaction of lymphocytes did not correlate with the rate of HD progression, or with the stage of the disease. Because of the relatively short ranges of CAG repeat expansion (median 45) in HD patients the rate of apoptotic change in lymphocytes could not be correlated reliably with the length of CAG repeat expansion.

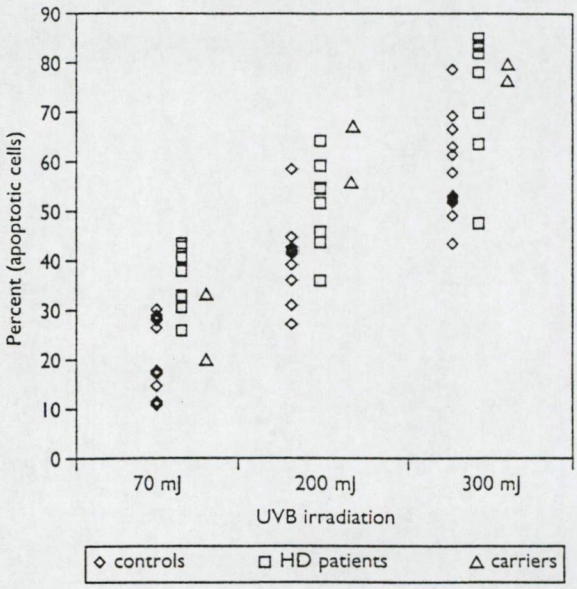

Fig. 1. 70, 200 and 300 mJ UVB irradiation-induced apoptotic cell death is significantly higher in eight HD patients and two asymptomatic carriers compared to ten normal controls. $p < 0.0171$ (70 mJ), $p < 0.0099$ (200 mJ), $p < 0.0126$ (300 mJ). The points represent the percentage of apoptotic cells from each individual.

DISCUSSION

There is a selective neuronal degeneration in the restricted brain regions in HD patients induced by mutant huntingtin. However, non-neural tissues are also involved in the pathological process, because huntingtin is widely expressed in the tissues. This is the second study, which proves that in HD not only cells of the central nervous system are prone to apoptosis, but also the peripheral lymphocytes [12]. We provide further evidence that increased susceptibility of HD cells to apoptosis is not restricted to neurons. We used UVB irradiation to induce apoptosis in lymphocytes. To monitor the effect immunohistochemical detection of Apo2.7 expression in irradiated cells was utilized. Sawa *et al.* [12] applied different methods (staurosporine exposure) for induction of apoptosis in cultured lymphoblasts. In their study the number of cells displaying apoptosis markers (DNA laddering, caspase assay, depolarization of mitochondria) was doubled in HD lymphoblasts cultures. In our present work the same tendency of susceptibility of CD3+ lymphocytes to differently induced apoptosis was noted. Both sets of experiments however substantiated that under different stress conditions higher number of HD lymphocytes react with apoptosis. Even having mutant huntingtin the HD lymphocytes function normally, because they have much shorter lifetime than neurons. The role of huntingtin in the apoptotic process is still not clear. Targeted disruption of HD gene in mice resulted in embryonic lethality and increased apoptosis in the embryonic ectodermal cells [13,14]. These data suggest that normal huntingtin has an anti-apoptotic effect. The lower incidence of cancer in HD patients, but not in their healthy relatives support the theory, that mutant huntingtin can protect against cancer inducing apoptosis in preneoplastic cells [15]. *In vitro* experiments showed that mutant huntingtin induced neurodegeneration by apoptotic mechanisms in cultured striatal neurons [16]. Expression of extended polyglutamin stretch of huntingtin itself is able to induce apoptosis in

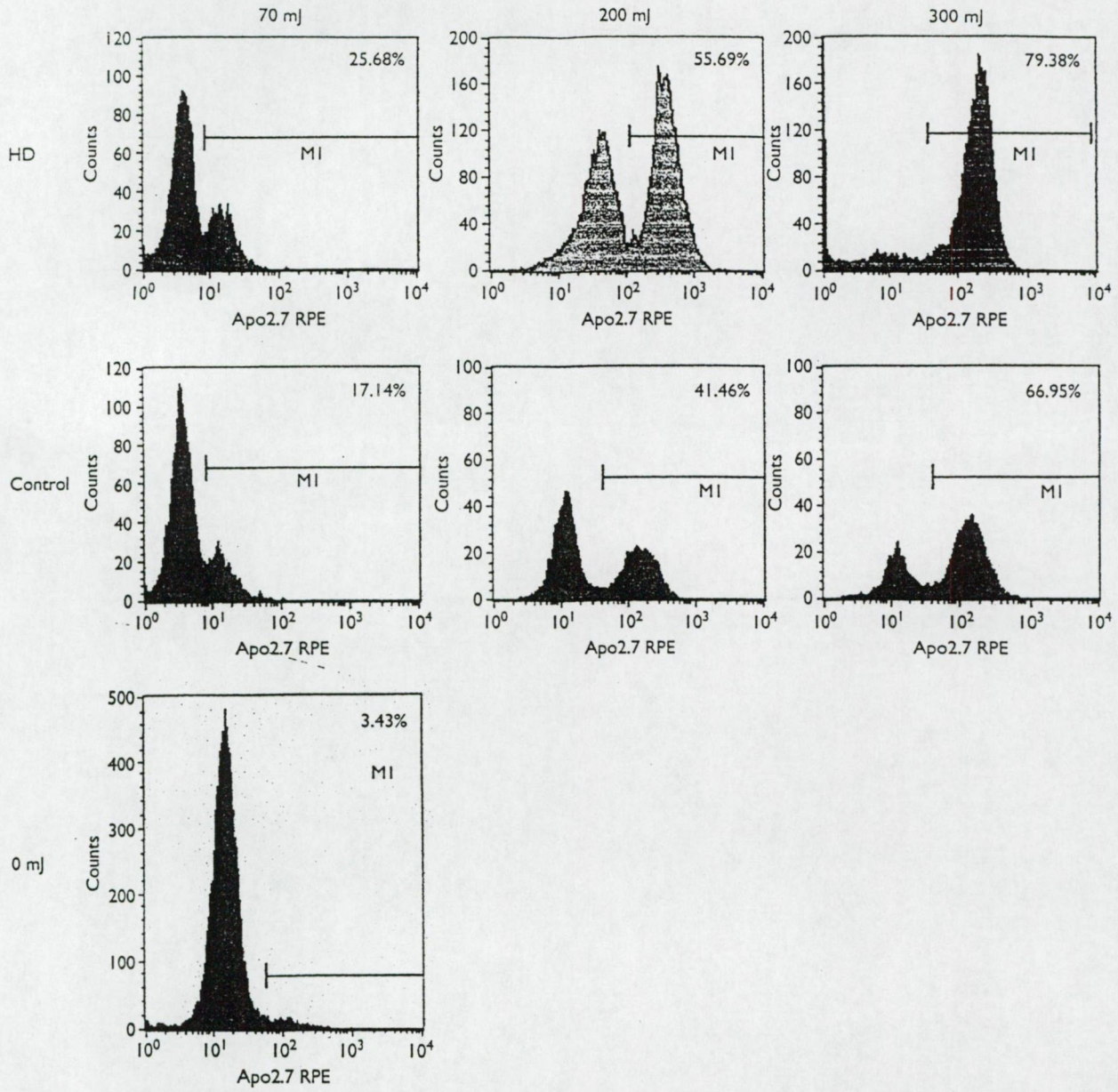

Fig. 2. Flow cytometric analysis of HD and control lymphocytes labelling with Apo2.7-PE antibody recognizing apoptotic mitochondrial membrane protein. Apoptosis was induced by 70 mJ, 200 mJ and 300 mJ UVB irradiation. The second profile indicates proportion of apoptotic cells within the M1 region. The UVB induced apoptosis is more enchanced in HD patients in each experimental paradigm.

cell cultures mediated by caspase 8,9,3 activation [17–19]. Mutant huntingtin may promote apoptosis in all cell type, but only a restricted neuronal subpopulation is the most sensitive to this process causing neurodegeneration.

CONCLUSION

Recently investigation has focused on the role of apoptosis in neurodegeneration. This study provide a further support of that, because, we found an increased susceptibility of HD lymphocytes to apoptosis. The enchanced apoptotic process may serve as a therapeutic target for the treatment of HD patients and anti-apoptotic factors/drugs such as caspase inhibitors, may prevent the asymptomatic carriers to develop the symptoms, helping to survive or preserve neurons.

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